AN EXPLORATORY INVESTIGATION OF THE SELECTIVE VALUE OF CERTAIN GENES AND THEIR COMBINATIONS IN DROSOPHILA [1]

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The viability and vitality of fruit flies have interested geneticists ever since certain mutant stocks were found to be weaker in a number of ways than the wild stocks from which they had arisen. As Timoféeff-Ressovsky (1934) states, vitality of an organism is an indefinite quality to measure. It consists of a number of different factors such as duration of life, fertility, adaptability, mortality rate, resistance to disease, immunity, etc., only a few of which can be weighed and compared objectively. Fertility has been studied in several different ways. Saveliev (1928) and Timoféeff-Ressovsky (1933) have compared the total number of eggs or offspring; Lüers (1935) has studied deviations from the expected 1 : 1 sex ratio; Timoféeff-Ressovsky (1933 and 1934) and Csik (1935) have examined the alteration of 1 : 1 back cross ratios; and Timoféeff-Ressovsky has also considered the mortality occurring between the egg and adult stages in the life history of *Drosophila*. Pearl, Parker, and Gonzalez (1923), Gonzalez (1923), and Lüers (1935) have studied life duration as a criterion of viability by comparing life spans of males and females together or separately in different stocks. Comparing the life durations of males with that of females of the same stock has been done by Pearl and Parker (1924) and Lüers (1935).

Gonzalez (1923) found that different combinations of the second chromosome mutations black, purple, vestigial, arc, and speck had different effects on the longevity of the flies, and that one mutant gene added to a poor stock might improve it considerably. Other reports of similar circumstances are: an example of the combination of black and curved producing greater numbers of offspring than either stock alone, found by Bridges and Sturtevant (1914); a "white" mutant produced by heat which showed greater viability than the wild from which it arose, very briefly reported by Jollos (1932); and a gene for trans-

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parent eye which, when combined with it, improved a weakening effect produced by a gene for eye color in *Ephestia*, investigated by Kühn (1932) and Kühn and Henke (1929–1932). Other instances of genes influencing viability in different ways and to varying degrees have been worked out for several different gene combinations. Timoféeff-Ressovsky (1934) worked on *Drosophila funebris*, and with the genes eversae, singed, venae abnormes, miniature, lozenge, and bobbed. Csik (1935) showed the interactions of Lobe^2, Minute-173, and plexus to produce differences in fertility in *Drosophila melanogaster*. Gonzalez (1923), Csik (1935), and Lüers (1935) have shown quite conclusively that the factors which influence fertility and longevity (viability) of flies are the manifestations of the already known genes and their interactions, not unknown lethal genes.

In 1933 I became interested in the question of whether such occurrences were common, or at all general, and set out to test several combinations of genes in *Drosophila melanogaster*. Because I was chiefly interested in the way these combinations of genes might influence the survival value of the flies, the experiments were arranged to show not only comparative fecundity in different genotypes, but also comparative rapidity of production.

It is well understood that the rapidity with which offspring are produced is of great importance for the survival of the species. In this study, rapidity of production was measured in terms of the length of time between generations. This is the time that elapses between a parent's reaching the reproductive period and the mean time at which all of her offspring become mature, and is a function of the number of individuals in the family and the period in the lifetime of the parent at which they were produced. Selective value of a genotype consists not only in rapidity of production, but in the size of the family as well. Consequently, these two variants have been expressed together in the term Selective Index (S). S is used to represent the value in an evolutionary sense of flies of each genotype; and since this value varies directly as the number of offspring they produce, and inversely as the length of time between generations, it is the ratio of the former to the latter (mean number of offspring/mean time between generations).

I wish to express the deepest gratitude to Professor A. F. Shull not only for suggesting the problem but for furnishing invaluable help throughout its progress.

Methods

Drosophila melanogaster was selected as the experimental animal, and in the three years during which the experiments were conducted, a

total of nineteen different mutations were examined. Which of the hundreds of known mutations were to be studied was decided largely by chance and to some extent by the results of Gonzalez's work (1923). The nineteen mutant stocks used were: black, brown, claret, Curly, cut^{6}, Dichaete, eosin, eyeless, glass, hairy, miniature, purple, roughoid, scarlet, sooty, vermilion, vestigial, white, and yellow. Flies were kept in 70-cc. vials at constant temperature of 25° C. and fed on standard cornmeal-agar-molasses food medium.

Sixteen experiments were completed between November, 1933 and January, 1937. For all of these except the third, stocks were prepared from three mutant genes and their various combinations. Thus eight stocks were used, consisting of three single mutant stocks, three with two mutant genes, one with three mutations, and wild as a control. Experiment 3 differed in that six different mutant genes were combined with vestigial singly, so that the eight stocks consisted of the vestigial stock and six stocks with two mutant genes one of which was vestigial, plus wild. Experiment 1 and its repetition 1*a* combined the genes *y, w,* and *Cy*; Experiment 2 combined the genes *bw, D,* and *st*; Experiment 3, the genes, *y, w, v, st, ca,* and *ey* individually with *vg*; Experiment 4, *vg, bw, D*; Experiment 5, *y, pr, vg*; Experiment 6, w^{e}, *vg*, e^{s}; Experiment 7, *y*, ct^{6}, *gl*; Experiment 8, *m, b, gl*; Experiment 9, w^{e}, *vg, gl*; Experiments 10 and 10*a*, w^{e}, *pr, vg*; Experiment 11, ct^{6}, *b, st*; Experiments 12 and 12*a, m, pr, st*; and Experiment 13, *ru, h,* e^{s}. Experiments 1, 10, and 12 were repeated because of what was believed to be abnormality in one of the eight stocks.

In October, 1934, a process of "purification" was begun in several of the stocks which were to be used later in the experimental work. The method was patterned after one recorded by Timoféeff-Ressovsky (1933 and 1934), the purpose of which was to get all the mutant genes into as nearly as possible the same genetic environment, so that all flies would be essentially similar in all genes except the one for which the stock was named. This process was maintained continuously until the end of the experimental work, and those flies used in making up stocks for individual experiments were taken from these purifying stocks at various times. Of the nineteen stocks named, brown, claret, Curly, Dichaete, eyeless, vermilion, and white were not purified at all before using. Several of the stocks were not added to the purification process until the summer of 1935. Consequently flies in different experiments were purified for different lengths of time, varying from five generations to fifty-three generations.

The procedure in a single experiment was to use five vials for each stock, each containing one female and two males not more than twelve

hours old. Single females were used rather than group matings, in order to show individual variations of the females. All forty vials were prepared and the flies added at the same time, in order to keep external modifying factors as nearly equal as possible. For the same reason the forty vials were kept in close proximity, and all flies were transferred at the same time. On the second day, fourth day, sixth day, etc., after the experiment was begun, all parent flies were transferred to new vials, until the females died. The original vials in an experiment were designated "*a*," the vials into which the flies were transferred the first time "*b*," the third vials in the series "*c*," and so on. If a male died he was replaced, so that there was the same number of flies in each vial at all times.

The technique used was not constant throughout the sixteen experiments. In the first eight experiments only one male was regularly used in each vial, but in Experiment 8 a second male was added to each vial in which no eggs had been produced by the eighth day. In all later experiments two males were kept in each vial, because it had been found that a female would sometimes mate with a second male when she had not successfully mated with the first one.

The first flies to emerge in a vial appeared sometimes on the eighth day after the parents were put into the vial, sometimes on the ninth, and often on the tenth day. All vials in one experiment were counted on the same day. Thus every vial was not counted on the first day that flies emerged therein. But after the first count flies were counted in each vial every forty-eight hours until five counts had been recorded. Some flies remained in the vials for two days (those that emerged later in the day on which the count was made), so that some of the population removed on the second day thereafter were old enough to have laid eggs. Consequently, there was the possibility of an F_2 generation emerging in the same vials any time after the tenth day.

It was determined by special treatment of the beginning of Experiment 9 that only 0.95 per cent of the flies emerging in the first four sets of vials were omitted by discontinuing counting after the fifth count. It is not likely that even this small percentage was omitted in all experiments or even later on in this particular one, because as parent flies grew older their offspring tended to emerge earlier, and also tended to be more concentrated in the first few counts, than earlier in the experiment.

Results

The age of each female at death, the number of her offspring, and the length of time between her generation and that of her offspring was

calculated, then a mean of each taken for each group of five females of the same genotype. For instance, the mean life span of the white-eyed females in the first experiment was 21.75 days, their mean production was 501.2 offspring, and the mean time between generations was 20.43 days. Those females that produced no offspring were included in the means although there is some possibility that they were not behaving as they would have done in a natural population. But since so little is known about the irregularities involved, and no special study of them was made in this research, it could not be assumed that they were abnormal enough to exclude them from comparison with others of their genotype.

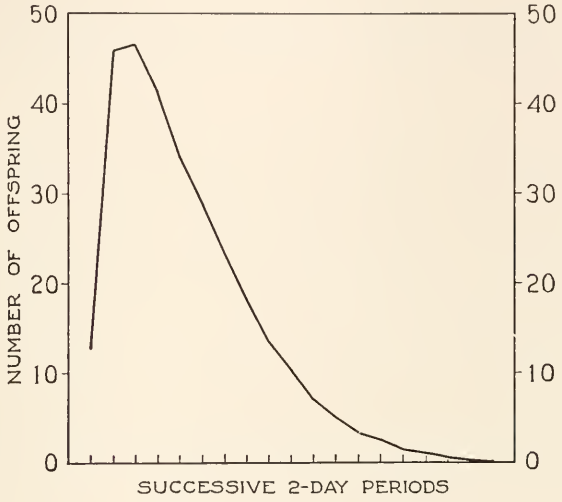

FIG. 1. Mean production curve of 593 females.

Because of accidental deaths and escapes the exact life span of some females was not known. In some instances in which a female's age could not be ascertained, her record up to the time of her escape showed that she would probably have produced no more offspring. There is a normal curve of production, which shows very clearly on the charts recording numbers of offspring in two-day periods. A rapid increase in the totals of the first few counts is followed by a gradual decline which may continue for many days, but gradually tapers off to zero. Figure 1 represents the mean of all recorded production curves. Several females laid eggs even after their production curve for offspring had dropped to zero, but these eggs did not hatch. In any case, if a female had not laid fertile eggs in three successive vials, her family was considered complete. It is well known that *Drosophila* females

often live beyond their egg-laying period (Hanson and Ferris, 1929; Shapiro, 1932). Very few of the flies in these experiments laid eggs in the last several days of their lives, and many of them lived for many days after egg-laying had ceased. The families of escaped females, if the production curve showed that they were complete, were considered comparable to those more completely known, and were included in the means. If, however, a female escaped during her productive period, it was assumed that her family was still incomplete, and the number of her offspring up to the time of her escape was not included in the mean of that group.

Individual females showed wide differences among themselves both in length of life and in numbers of progeny. In any one experiment females supposedly identical genetically showed wide variations, and the same is true of females of the same genotype from different experiments.

A source of error in comparing the production of individual females may be in assuming that they all mated equally and that the mate of each was equally capable of fertilizing all of her eggs. Guyénot (1913) showed that when a female's supply of spermatozoa is exhausted, her egg-laying stops for a time and then may continue, but unless a new mating is accomplished these eggs are infertile. Castle, Carpenter, Clark, Mast, and Barrows (1906), Hyde (1914 and 1924), and Lutz (1914) also found that a female must mate more than once if all her eggs are to be fertile. The females in this investigation, at least those prior to Experiment 9, were under unnatural circumstances in so far as mating possibilities were concerned, because if their single mates were incapable of fertilizing all their eggs, their families would be smaller than they would have been if new matings could have been made later in life. There are only three females from these experiments which were obviously affected in this way, though there were probably others affected to some lesser degree. In the first experiment no males were replaced. Consequently the drop in a female's production after the death of her mate can be demonstrated. Table I presents the number of offspring produced in successive vials, by three females whose mates died early in their lives, compared with the mean number of offspring for the same period, from their sisters whose mates survived. The means were computed from all females of one genotype which had mates; consequently the three named females were included in the means until they were without mates. Means in whole numbers were considered accurate enough for the purpose of Table I. The heavy vertical lines dividing the three families represent the points at which the males died. After this time the female was alone in the successive vials. It

will be seen that the number of her offspring in the two-day period directly following the death of her mate was for all three females normal. That is, the reduction in family size did not begin to manifest itself until the third day after the absence of the male. None of the females from which the means were drawn had as short productive periods as the three illustrated. One of the yellow white females ceased production in the "l" vial, and the other white and Curly females all continued producing at least through the "i" vials.

Many females went on producing offspring at the normal rate after their mates had died and had been replaced. Several females' production diminished in the counts immediately following such a change, then rose again to the level that was expected for their stage in the production curve. The explanation is probably that the female did not mate immediately with the new male. When a new male was added to a

TABLE I

Vial of Series	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	*l*	*m*	*n*
White ♀ 5	45	96	89	100	63	52	0	0	0	0	0	0	0	0
Mean	36	82	80	76	52	62	54	49	30	9	1	1	1	0
Curly ♀ 5	19	72	89	65	12	1	0	0	0	0	0	0	0	0
Mean	15	76	82	67	63	66	57	53	32	28	40	37	30	7
Yellow white ♀ 2	4	52	68	60	65	58	55	27	6	4	2	0	0	0
Mean	11	59	74	63	71	54	56	48	42	29	29	18	8	4

vial to replace a loss by death and the female's production decreased, just as happened in the three cultures illustrated, it could be surmised that they had not mated. In Experiment 3, one of the vestigial eyeless females illustrates a similar phenomenon. She laid no eggs during the first eight days of the experiment, but when a new male was added she produced 86 offspring within the next six days and continued thereafter in quite a normal way. In several vials in which the flies proved entirely sterile they were seen mating, so the fault was not always merely that they did not mate. These illustrations all contribute to the probability that the cultures were not all comparable in this regard, and that a number of the 99 females which were apparently sterile might have become productive if a suitable male had been present.

In determining the number of offspring produced by each female, no record was made of those that died in any of the immature stages

of development. Those which did not live to reach the reproductive period could have no influence on the length of time between generations. Consequently, differences between the families of different females rest only on those offspring which reached adulthood.

Comparisons of Wild Females and Vestigial Females

The age records of the 75 wild females studied in this investigation varied from 3.25 to 57.75 days. The mean age at death of all 75 was 26.21 ± 1.42 days. Mean life spans of wild-type females of stocks used by other *Drosophila* workers have been reported as follows: Hyde (1913), 34.5 days (from a study of 97 females); Lutz (1914), 26.4 days (unmated females); Pearl and Parker (1922), 38.8 days ("expectation of life"); Gonzalez (1923), 40.62 ± 0.42 days; Pearl and Parker (1924), 48.0 days; Alpatov (1930), 49.11 ± 0.50 days; Gowen (1931), 33.1 ± 0.6 days; Lüers (1935), 20.1 ± 0.5 days. Pearl and Parker (1922) isolated several different "lines" of wild flies and found their longevity to differ considerably (from 22.04 ± 1.57 to 50.02 ± 0.85).

The ages of the 34 vestigial females varied from 47.75 days to 1.25 days, and the mean was 20.26 ± 1.63. Gonzalez (1923) found the mean of vestigial females from his stock to be 20.98 ± 0.4; Pearl and Parker (1924) found it to be 19.8 days; Hanson and Ferris (1929) found 20.13 days; Lüers (1935), 18.9 ± 0.2 days; Carver (1937), 28 days (average of 51 females). The numbers of females of other mutant stocks were not large enough to be employed in such comparisons; moreover, no other stocks have been measured as frequently by other workers as have these two.

Selective Index

The number of parent flies of each genotype in these experiments is too small to be used in computing reliable means either of fecundity or of longevity. Consequently, although the mean number of offspring is used in deriving the selective index, this figure must be considered as applying only to these particular flies and not to flies of these genotypes in general. The means used in calculating S for all the genotypes that were tested are those of fecundity and of time between generations, already mentioned.

The selective indices of all groups of flies which involved a particular gene and its combinations with other genes have been selected from the sixteen experiments and treated in such a way as to isolate the effect of that gene. To use the gene for black as an example, the S of the

black flies was compared with the S of the wild control flies. When such a comparison was made in Experiment 8, S for black flies proved to be 2.01 times S for wild. These two stocks are supposedly identical except for the genes at the black locus. This comparison, then, should show whether the mutation from b^+ to b is of survival value to the flies. But in Experiment 11 the index for the same mutation (b) was only 0.14 times that for the wild flies. Such a discrepancy is probably caused by the individual differences of the twenty females involved.

Similarly the S of miniature black flies can be compared with the S of miniature flies, which are presumably identical except for the gene

TABLE II

Mean ratios of selective indices for all genes studied, arranged in order of magnitude

Gene	Mean Ratio of S's	No. of Ratios of Which it is the Mean
Cy	2.57	8
w	2.14	9
h	1.80	4
w^e	1.76	16
pr	1.75	18
st	1.65	14
v	1.57	1
gl	1.41	11
vg	1.15	25
D	1.14	8
ey	1.09	1
ru	1.08	4
ca	1.04	1
y	0.95	17
bw	0.95	8
m	0.65	11
e^s	0.60	8
b	0.36	8
ct^6	0.25	8

at the locus b. All such comparisons of flies carrying the gene b with flies identical except for this one gene were grouped together and the mean derived. The mean of all ratios comparing b flies with b^+ flies is taken as the value of b in comparing it to the other mean ratios of S's, derived by a similar process, for each of the other genes used. Flies carrying the genes *v*, *ca*, and *ey* could be compared only once to flies with their wild-type alleles because they were used only in Experiment 3 which was carried out differently from the rest. Consequently their values, derived from ratios of S's, are hardly comparable with those of the other sixteen genes.

In Table II are summarized all the mean ratios for S arranged in order of magnitude. *Ca*, *ey*, and *v* are included for the sake of com-

pleteness. Curly appears at the top of the scale, but it should be pointed out that it was tested in only two experiments (1 and its repetition 1*a*), and that in one of these the yellow flies were abnormally poor (compared to their performance in other experiments), making the ratio of *y Cy* to *y* flies very high. If this one ratio is omitted, and the mean derived without it, Curly drops to 1.29. White likewise is raised to its high rating because of the defective yellow flies in Experiment 1. Omitting that one ratio of 10.42, the mean ratio of white falls to 1.11. Hairy is even less secure than these two in its position, because its mean is drawn from only four ratios, all in Experiment 13. In the strictest sense, brown, claret, Curly, Dichaete, eyeless, vermilion, and white should not be compared on the same scale with the genotypes that had been purified. But eosin, purple, glass, miniature, sooty, and cut[6] at least, are comparable for the purpose of this table, and scarlet, vestigial, and yellow also, except for a few of their ratios which involve unpurified genes.

Gene Combinations

A few genes have been brought to light whose interactions have a decided effect on the survival value of their bearers. Scarlet and purple are the most striking example of this phenomenon. In Experiments 12 and 12*a* their effects, measured by their selective indices, are parallel:

Exp.	*pr*	*st*	*pr st*
12	15.34	7.80	39.42
12*a*	19.46	4.59	36.35

Thus the value of the two genes together seems to be far superior to either one alone. Other examples of the same type of interaction are: *y* and *Cy* in Experiment 1, *y* and *w* in Experiment 1, *vg* and *bw* in Experiment 4, *y* and *pr* in Experiment 5, *vg* and *gl* in Experiment 9, and *m* and *st* in Experiments 12 and 12*a*. It should be pointed out that in the repetition of Experiment 1 the interactions of *y*, *w*, and *Cy* are not at all parallel to those given for Experiment 1, in which it is believed that the yellow flies were defective.

The following are combinations in which a third gene, interacting with a combination of two, brought about a higher *S* than either genotype had before being combined: *y Cy* and *w* in Experiment 1*a*, *bw D* and *vg* in Experiment 4, w^e *vg* and *pr* in Experiment 10*a*, and *ru h* and e^s in Experiment 13. The examples from Experiment 1*a* and 10*a* are not substantiated in their parallel experiments, 1 and 10.

There are many more examples from these experiments in which a gene with low selective value is combined with one of higher value, the interaction producing a selective value intermediate between the two: *y* and *Cy* in Experiment 1*a*, *w* and *Cy* in Experiment 1*a*, *vg* and *D* in Experiment 4, *vg* and *pr* in Experiment 5, *vg* and w^e in Experiment 6, e^s and w^e in Experiment 6, *y* and *gl* in Experiment 7, *vg* and w^e in Experiment 9, *gl* and w^e in Experiment 9, *pr* and *vg* in Experiment 10, *pr* and w^e in Experiment 10*a*, ct^6 and *st* in Experiment 11, *b* and *st* in Experiment 11, *m* and *pr* in Experiment 12*a*, e^s and *h* in Experiment 13, and e^s and *ru* in Experiment 13. There are several examples of the latter type of reaction in which a third gene is added to a combination of two, the resulting flies being intermediate in selective value.

There are a great many examples of flies which had definitely lower value brought about by the interaction of genes. In this class belong not only flies carrying two mutant genes whose selective value was lower than flies carrying only one of the two, but those whose value was lower than a combination of two when a third gene was added. This type of interaction may be found in practically every experiment.

Combinations of scarlet and purple, brown and vestigial, and yellow and purple seem to be the most advantageous to flies in so far as their survival value is concerned. Scarlet and purple were combined with the same favorable results in two experiments, whereas the other two combinations were tested only once. In combinations of genes which result in flies intermediate in *S* between the values of the components, we may look upon one as an advantageous influence and the other disadvantageous. Thus eosin, purple, and scarlet, and less often glass and Curly seem to belong in the category "advantageous influence," and black, miniature, cut^6, sooty and less obviously yellow and vestigial seem to be most often "deteriorating influences."

Discussion

Interaction of Genes

In this investigation there is some evidence that certain combinations of two genes produce flies of higher selective value than does either gene alone. If such indications can be seen from a study in which only nineteen genes were tested, it must be a rather general phenomenon, and a continuation of this sort of study should uncover more such circumstances. In this preliminary exploration of the field, at least three genes have been found that seem to carry their "good" influence into combinations with genes of weakening influence, and to

bring about improvement in survival value of those flies. Purple in Experiment 5 and eosin in Experiment 6 are the most outstanding examples of this phenomenon. In Experiment 9 eosin, in combination with vestigial and glass, stands out as an advantageous influence again; and in Experiments 12 and 12*a* purple shows the same effect. But in Experiments 10 and 10*a* purple seems to be a weakening influence, and vestigial advantageous. Eosin in combination with vestigial, here, is very much lower than either alone. Whether this indicates that eosin is erratic in its "good" influence, or that something had happened to the purple stock, or whether all stocks were in a state of depression at the time these experiments were in progress, is not known, but it is true that two of the lowest values of S for the wild control stocks occurred in these two experiments (as shown in Fig. 3).

Glass, tested in three experiments, is sometimes in the rôle of "good" influence, sometimes not. Its advantageous tendency seems to be overbalanced by the damaging influence of the genes black, cut^6, and miniature. Scarlet likewise is not at all consistent, but does exert an advantageous influence on these three detrimental genes. Curly, although tested with only white and yellow, does seem to show promise of combining to good advantage.

It is interesting to note that these genes which have just been mentioned as the most advantageous influences discovered, at least in these experiments, are the same genes that appear in the majority of the upper ranks of Table II. Their high standing in that scale signifies that flies with these genes have a better chance of survival in an evolutionary sense, than do flies without these genes.

Vestigial, in all tests except those of Experiments 10 and 10*a*, appears to be a degrading influence, and almost every gene combined with it induced improvement in the combination. Black, cut^6, miniature, and sooty give promise of nothing but detrimental influence on other genes, and are practically lethal when combined. These are also the lowest four genes as listed in Table II.

Variability

Several factors dictate caution in the drawing of conclusions from the results of this research. The most outstanding hindrance is the variability of the flies. Females of the same genotype not only varied widely in their duration of life, but in their production, and in several factors connected with production.

(1) *Numbers of Offspring.*—Nonidez in 1920 wrote, "the rate of egg laying is variable in different females," and Shapiro (1932) ob-

served that "life span, egg laying period, and total egg output varied greatly from individual to individual in the various crosses." Although these were studies of egg production the same variation exists when reckoned in adult offspring. For the 647 females studied in this research, environmental conditions such as temperature, moisture, age of food, amount and consistency of food, light, ventilation, density of population (except for the difference of one male in about half the vials), etc., were as uniform as it was possible to make them, yet in only seven instances did two females of the same genotype in any one experiment have equal life spans and at the same time produce approximately equal numbers of offspring. Among 34 other cases in which two (or three) females of the same genotype lived equally long (ex-

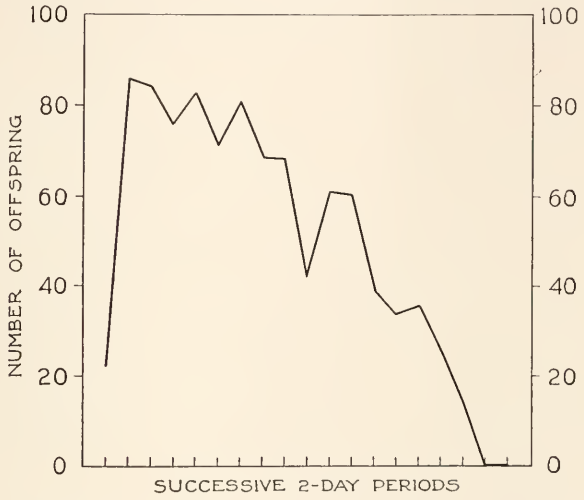

FIG. 2. Offspring production curve of a wild female from Experiment 4.

cluding pairs of which one member was sterile), differences in production were considerable. The difference in output between the two individuals of any of these pairs varied from less than 100 to nearly 700, and the difference was as variable and as great among the shorter-lived pairs as among the longer-lived ones.

Individual females produced widely different numbers of offspring in successive periods of equal length. This form of variation has been referred to by other *Drosophila* workers in the past. Hyde (1913) reported cyclic production of individual females. Hanson and Ferris (1929) had evidence of somewhat the same phenomenon in six of their females. Their graphs of daily egg production show the same irregularities of outline from day to day that my graphs show for production

of imagos. A curve such as that in Fig. 1, drawn for any one female, shows great irregularities in height for successive two-day periods (see Fig. 2). Dobzhansky (1935) wrote that "the number of eggs deposited in the same culture on successive days is notoriously variable," but his study was of groups of flies, not individual females.

(2) *Variation of a Single Stock in Several Experiments.*—These individual variations are probably the chief reason for the difference found between groups of five females of the same genotype in different experiments. A graphic demonstration of this is the comparison of S of wild control stocks in all sixteen experiments. In Fig. 3 the S's are plotted as ordinates and the experiments arranged in their chronological order from left to right, spaced in their proper seasons. This arrange-

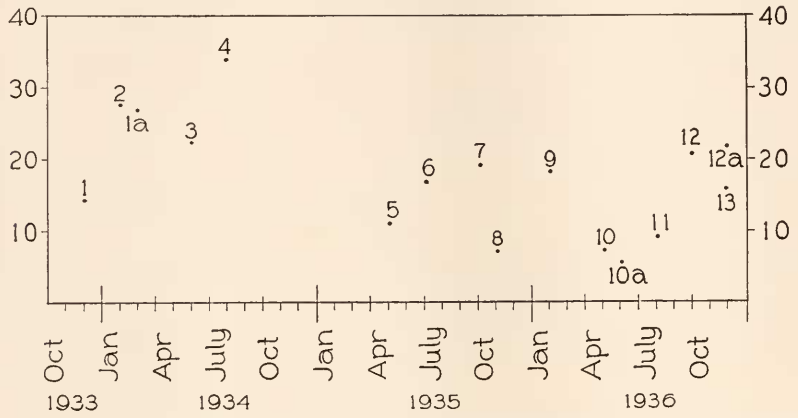

Fig. 3. Selective index of the wild flies in each of the sixteen experiments spaced chronologically.

ment has been used in order to show that in so far as can be judged from a small number of experiments, seasonal variations were not the cause of the fluctuations in S. It can be seen that the highest S of all occurred in Experiment 4 which was conducted in midsummer, yet three of the lowest values of S are from Experiments 10, 10*a*, and 11 which were also summer ones. Likewise both high and low S's occurred in fall and winter seasons.

(3) *Time of Development.*—Another form of variability occurred in the time of development of the flies. The offspring counted in these experiments developed from eggs that were laid in 48-hour periods, yet the imagos emerged over a period varying from eight days after egg-laying began in the vial, to as much as twenty-four days. The latter time interval between egg-laying and emerging of the adult could only

be measured in the specially treated first part of Experiment 9, where the late emerging offspring were saved and recorded. In all other experiments it could only be certain that offspring were emerging over a period as long as ten days.

(4) *Vigor.*—Variations in life span were often dependent upon the vigor of the flies. Those that were weaker and less active became more often caught in the food, and died. The more active ones spent more time on the stopper and dry sides of the vial and hence had less opportunity of sinking into the sticky food.

Inequality of Stocks in Amount of Purification.—It was not feasible to purify all stocks equally before using them in the experiments. Since no stocks had been purified before this study was undertaken, it would have entailed too long a delay before the work could be launched, if it had been decided to wait until all stocks were sufficiently pure. All through the experimental work tests were being performed to see whether certain genes could be used together, and until it was known whether a stock could be used, in most cases, it was not purified. The purification process itself involved so much labor that no more stocks could be maintained than were necessary. Consequently some of the stocks, those in the first several experiments, were not purified at all, and others for different lengths of time. This inequality of stocks detracts somewhat from their value in comparisons.

Comparative Reliability of the Several Factors

Since selective index is theoretically the correct measure of the success of one genotype in comparison to others in an evolutionary sense, it is well to consider whether fecundity, length of time between generations, or length of life might be substituted for S without serious reduction in reliability of the tests. Since S takes into account not only the number of offspring produced, but also the rapidity with which they are produced, it is logically superior to any form of evaluation which embraces only one of these qualities. Life duration as a measure of advantage or survival value has no correlation with S. This is to be expected since length of life has no necessary bearing either on the number of offspring or on their rapidity of production. There were several females in these experiments which lived longer than average, yet produced few or no offspring.

Time between generations, although the values for it are statistically the most reliable of any of the four criteria, is not by itself a valid measure of survival value because it often happens that a group of flies which has a high mean number of offspring will also have a relatively

long mean time between generations. This large total of progeny is apt to be distributed over a longer period than is true of another group which has small productive capacity. That is, the general shape of the production curve for the two groups is the same at the beginning (the rapid rise in the first few periods), but for the low fertility group the height of the curve usually decreases soon thereafter, gradually and irregularly, while the curve of the high fertility group not only goes higher usually, in the first few counts, but stays high for a longer time before declining, or it may decline then rise again to a lesser peak. The decline is usually more gradual than in the lower fertility group, thus making the mean time of production longer. Thus a genotype which had the lowest mean time between generations in one experiment often was the least productive stock, and although all of the offspring were produced early, there may have been more offspring of another stock at that same time, even though the mean time between generations of that other population is longer.

The selective index, which has been introduced as a means of measuring the value of genes to flies in their survival in an evolutionary sense, is more reliable in evaluating the success of different types in survival than any of the heretofore measured viability factors. It is a numerical value and in order to use it for comparisons between genotypes, large numbers of flies must be raised and tabulated, and there remains a great deal to be done before wholly reliable conclusions regarding the comparative selective values of any of the *Drosophila* genes can be estimated. I consider the evidence that this investigation offers as being at least indicative of what sorts of things may be found when more reliable bases of comparison are obtained.

Summary

1. A total of 203,159 flies (offspring) were counted in this investigation. Of this number, 190,318 were the basis of the selective index calculations. (The remaining 12,841 offspring were in families which were not comparable with the others for several reasons.)

2. In sixteen experiments a total of 647 females were compared, in groups of about forty, in respect to longevity, fecundity, and rapidity of production.

3. A wild-type female in Experiment 7 had the longest life span of all (57.75 days), and the longest mean duration of life of any one group was exhibited by the five wild females of that same experiment (42.25 days). Other groups which ranked comparatively high in longevity were the yellow cut[6] females of Experiment 7, the eosin ones of Experiment 6, and the yellow white ones of Experiment 1*a*.

4. The highest fertility record (taking into account only adult offspring) was exhibited by one of the eosin females in Experiment 6 (1434 offspring). The highest mean number of offspring of any group was found in the group of which this female was a member, the five eosin females of Experiment 6 (1046 offspring). The yellow purple females of Experiment 5 also showed high fecundity.

5. Selective index, the value of an individual in selection, is calculated by dividing the mean number of offspring by the mean number of days between generations. This is logically a better criterion of value to a race than is either longevity or fecundity.

6. Nineteen genes and their combinations, mostly in groups of three, were compared in respect to selective index.

7. In general, the addition of a mutant gene to another mutant gene or to a combination of mutant genes may (1) raise the selective value of the final combination above the value of either of the component mutations or combinations taken singly; (2) result in a selective value intermediate between those of the component mutations or combinations; (3) yield a selective value lower than the lowest of the components; or (4) make the selective value practically equal to that of one of the components. Some of the more striking examples of these reactions are mentioned below.

8. Flies which carried eosin, in combination with practically any other gene tested, had greater survival value than flies with the same genetic composition except for eosin.

9. Purple and scarlet genes are likewise advantageous, but less consistently so than eosin.

10. The genes cut^6, black, miniature, sooty, and vestigial have been demonstrated to produce a weakening effect when combined with several other genes separately.

11. It has been shown that females go on producing offspring at a normal rate for at least two days after the removal of their mates. Thereafter the number of offspring falls off rapidly.

12. Variability of females of the same genotype in production, longevity, vitality, and the time of development of their offspring renders uncertain the application of the conclusions of this investigation to *Drosophila* females in general.

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